

HIV-1 Co-Receptor Detection Set

CATALOG No.: PSI-1818

BACKGROUND:

Human immunodeficiency virus (HIV-1) and related viruses such as HIV-2 and SIV require coreceptors to infect target cells. The G protein-coupled receptors CCR3, CCR5 and CXCR4 are the principal receptors for HIV fusion and entry of target cells (1-4). CCR3 and CCR5 promote efficient infection of microglia, the major target cells in the CNS (3,4). Eotaxin, the CCR3 ligand and an anti-CCR3 antibody inhibited HIV infection of microglia. CCR5 is a principal coreceptor for macrophage- and dual-tropic HIV-1 strains fusion and entry of human white blood cells (2,5). The amino-terminal domain and the extracellular loops of CCR5 serve as HIV binding sites (6,7). High levels of CCR3 and CXCR4 expression were found on the neurons from both the central and peripheral nervous systems (8). CXCR4 is a principal coreceptor for T-cell tropic strains of HIV-1 fusion and entry of human white blood cells (1). CXCR4 is also required for the infection by dual-tropic strains of HIV-1 and mediates CD-4 independent infection by HIV-2 (9,10). CXCR4 associates with the surface CD4-gp120 complex before HIV enters target cells (11). Antibodies to CXCR4 block HIV-1 and HIV-2 fusion and infection of human target cells (10,12). The amino-terminal domain and the second extracellular loop of CXCR4 serve as HIV binding sites (12,13). Another G protein-coupled receptor CCR8 was also found to serve as a coreceptor for diverse T-cell tropic, dual-tropic and macrophage-tropic HIV-1 strains (14). CCR8 mediates CC chemokine I-309 induced monocyte chemoattraction and HIV-1 envelope fusion and virus infection, which can be prevented by the CCR8 ligand I-309 (15). Other proteins that can function as viral cofactors include Bonzo, CX₃CR1, DC-SIGN, and GPR15 (16-19).

KIT CONTENTS:

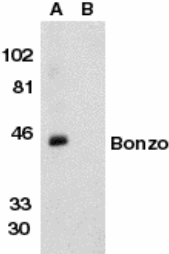
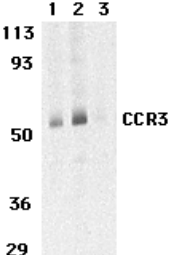
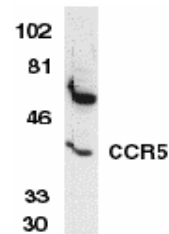
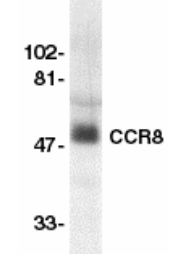
Bonzo antibody (NT2), **Catalog No. 2209 (50µg)**.
 CCR3 antibody (CT), **Catalog No. 1105 (50µg)**.
 CCR5 antibody (NT), **Catalog No. 1112 (50µg)**.
 CCR8 antibody (EL), **Catalog No. 2097 (50µg)**.
 CX₃CR1 antibody (NT), **Catalog No. 2093 (50µg)**.
 CXCR4 antibody (NT), **Catalog No. 1009 (50µg)**.
 DC-SIGN antibody (ED), **Catalog No. 2349 (50µg)**.
 GPR15 antibody (NT), **Catalog No. 2101 (50µg)**.

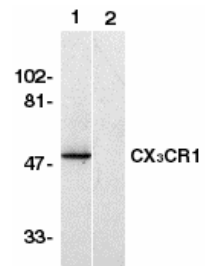
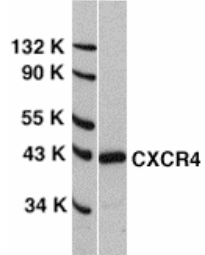
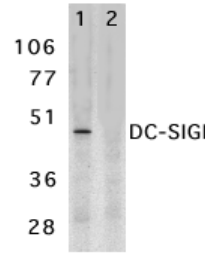
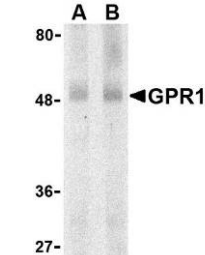
SOURCE:

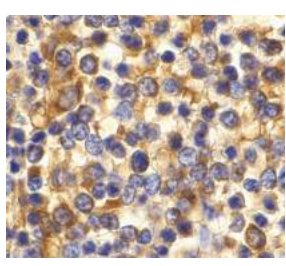
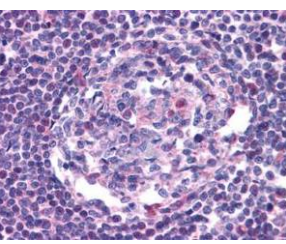
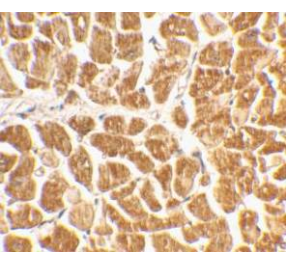
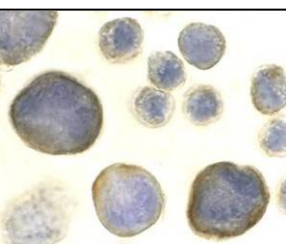
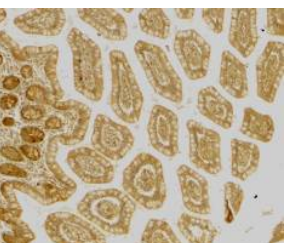
Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins.

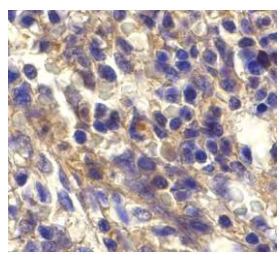
STORAGE:

Antibodies are supplied as affinity chromatography purified IgG in PBS containing 0.02% sodium azide. Antibodies should be stored at -20°C.

	<p>Western blot analysis of Bonzo in human spleen tissue lysate in (A) the absence or (B) presence of peptide with Bonzo antibody at 1 µg/ml.</p>
	<p>Western blot analysis of CCR3 in human spleen tissue lysates with CCR3 antibody at 1 (lane 1) and 2 µg/ml (lane 2), and 2 µg/ml in the presence of blocking peptide (lane 3).</p>
	<p>Western blot analysis of CCR5 in THP-1 whole cell lysate with CCR5 antibody at 0.5 µg/ml.</p>
	<p>Western blot analysis of CCR8 in human spleen lysate with CCR8 antibody at 1 µg/ml.</p>

	<p>Western blot analysis of CX₃CR1 in human spleen lysate with CX₃CR1 antibody at 1 µg/ml in the absence (lane 1) or presence of blocking peptide (lane 2).</p>
	<p>Western blot analysis of CXCR4 in HeLa whole cell lysate with CXCR4 antibody at 0.5 µg/ml.</p>
	<p>Western blot analysis of DC-SIGN expression in human placenta tissue lysate in the absence (lane 1) and presence (lane 2) of blocking peptide with DC-SIGN antibody at 2 µg/ml.</p>
	<p>Western blot analysis of GPR15 in human spleen lysate with GPR15 antibody at (A) 0.5 and (B) 1 µg/ml.</p>

	<p>Immunohistochemistry of CCR3 in human spleen tissue with CCR3 antibody at 10 µg/ml.</p>
	<p>Immunohistochemistry of CCR5 in human lymph node tissue with CCR5 antibody at 20 µg/ml</p>
	<p>Immunohistochemistry of human heart tissue using CX₃CR1 antibody at 2 µg/ml.</p>
	<p>Immunocytochemistry of CXCR4 in HeLa cells with CXCR4 antibody at 2 µg/ml.</p>
	<p>Immunohistochemistry of DC-SIGN in human small intestine tissue with DC-SIGN antibody at 10 µg/ml .</p>

	<p>Immunohistochemistry of Bonzo in human spleen cells with Bonzo antibody at 20 µg/ml.</p>
---	---



APPLICATION:

These polyclonal antibodies can be used for detection of Bonzo, CCR3, CCR5, CCR8, CX3CR1, CXCR4, DC-SIGN and GPR15 by immunoblot at 0.5 – 2 µg/ml. Some of these antibodies can also be used at 1 – 20 µg/ml to detect their respective proteins via immunohistochemistry or immunocytochemistry. **For research use only.**

POSITIVE CONTROLS:

Bonzo antibody: Human Spleen Tissue Lysate, **Catalog No. 1306.**
CCR3 antibody: Human Spleen Tissue Lysate, **Catalog No. 1306.**
CCR5 antibody: THP-1 Cell Lysate, **Catalog No. 1208.**
CCR8 antibody: Human Spleen Tissue Lysate, **Catalog No. 1306.**
CX3CR1 antibody: Human Spleen Tissue Lysate, **Catalog No. 1306.**
CXCR4 antibody: HeLa Cell Lysate, **Catalog No. 1201.**
DC-SIGN antibody: Human Placenta Tissue Lysate, **Catalog No. 1309.**
GPR15 antibody: Human Spleen Tissue Lysate, **Catalog No. 1306.**

RELATED PRODUCTS:

Bonzo peptide (NT2), **Catalog No. 2209P.**
CCR3 peptide (CT), **Catalog No. 1105P.**
CCR5 peptide (NT), **Catalog No. 1112P.**
CCR8 peptide (EL), **Catalog No. 2097P.**
CX3CR1 peptide (NT), **Catalog No. 2093P.**
CXCR4 peptide (NT), **Catalog No. 1009P.**
DC-SIGN peptide (ED), **Catalog No. 2349P.**
GPR15 peptide (NT), **Catalog No. 2101P.**

REFERENCES:

1. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 1996; 272:872-7.
2. Deng H, Liu R, Ellmeier W, et al. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996; 381:661-6.
3. Choe H, Farzan M, Sun Y, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996; 85:1135-48.
4. He J, Chen Y, Farzan M, et al. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* 1997; 385:645-9.
5. Dragic T, Litwin V, Allaway GP et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 1996; 381:667-73.

6. Doranz BJ, Lu ZH, Rucker J, et al. Two distinct CCR5 domains can mediate coreceptor usage by human immunodeficiency virus type 1. *J. Virol.* 1997; 71:6305-14.
7. Dragic T, Trkola A, Lin SW, et al. Amino-terminal substitutions in the CCR5 coreceptor impair gp120 binding and human immunodeficiency virus type 1 entry. *J. Virol.* 1998; 72:279-85.
8. Zhang L, He T, Talal A, et al. In vivo distribution of the human immunodeficiency virus/simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. *J. Virol.* 1998; 72:5035-45.
9. Doranz BJ, Rucker, Yi Y et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 1996; 85:1149-58.
10. Endres MJ, Clapham PR, Marsh M, et al. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell* 1996; 87:745-56.
11. Lapham CK, Ouyang J, Chandrasekar B, et al. Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. *Science* 1996; 274:602-5.
12. Brelot A, Heveker N, Pleskoff O, et al. Role of the first and third extracellular domains of CXCR-4 in human immunodeficiency virus coreceptor activity. *J. Virol.* 1997;71:4744-51.
13. Lu Z, Berson JF, Chen Y, et al. Evolution of HIV-1 coreceptor usage through interactions with distinct CCR5 and CXCR4 domains. *Proc. Natl. Acad. Sci. USA* 1997; 94:6426-31.
14. Goya I, Gutierrez J, Varona R, Kremer L, Zaballos A, Marquez G. Identification of CCR8 as the specific receptor for the human beta-chemokine I-309: cloning and molecular characterization of murine CCR8 as the receptor for TCA-3. *J. Immunol.* 1998; 160:1975-81.
15. Horuk R, Hesselgesser J, Zhou Y, et al. The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV-1 strains. *J. Biol. Chem.* 1998; 273:386-91.
16. Alkhatib G, Liao F, Berger EA, et al. A new SIV co-receptor, STRL33. *Nature* 1997; 388:238.
17. Combadiere C, Gao J, Tiffany HL, et al. Gene cloning, RNA distribution, and functional expression of mCX3CR1, a mouse chemotactic receptor for the CX3C chemokine fractalkine. *Biochem. Biophys. Res. Commun.* 1998; 253:728-32.
18. Pohlmann S, Baribaud F, Lee B, et al. DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J. Virol.* 2001; 75:4664-72.
19. Heiber M, Marchese A, Nguyen T, et al. A novel human gene encoding a G-protein-coupled receptor (GPR15) is located on chromosome 3. *Genomics* 1996; 32:462-5.

WESTERN BLOT PROTOCOL:



- 1) Load 20 to 25 micrograms of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2) Run at 20 mA per gel until the dye front is close to the bottom.
- 3) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 h, depending on the size of the target protein.
- 4) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:20,000 in blocking buffer) for 1 h in blocking buffer at RT.
- 8) Wash 3 x 10 min in washing buffer with shaking.
- 9) Drain washing buffer, add ECL solution and develop for 1 min.
- 10) Expose to X-ray film for 1 to 30 min.

MATERIALS NEEDED:

Nitrocellulose membrane
Non-fat dry milk
Tween-20
Antibody detection kit

TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS